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MORPHOLOGICAL CHARACTERIZATION, MULTIVARIATE ANALYSIS AND MICROPROPAGATION OF HYBRID ROSE (*Rosa indica* L.) GERMPLASM

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Rose is a highly economically important ornamental plant being globally used for aesthetic purposes since ancient times. In Pakistan, during last seventy years hundreds of Hybrid varieties were introduced and adopted for cut-flower production and landscape beauty. Several spontaneous mutants may also have originated during clonal propagation. In 2008, a comprehensive survey of Punjab areas was conducted and several hybrids were collected and planted in semi sub-tropical area (Rawalpindi/Islamabad). The varieties with stable phenotypic characters were further subjected to morphological analysis following UPOV-Rose descriptors during 2017 to 2019. Morphological diversity was analyzed using principal component analysis (PCA), cluster analysis and a large ratio of the characters was considered as dominant. The narrow range of variation was found within germplasm of Hybrid accessions and traits (11.43% and 10.14%) on factor plains (Factor 1 × Factor 2). The leading accessions 33, 34 and 6 were selected for their desirable plant attributes such as better flower size (cm), stem length (cm) and early flower production. Establishment of *in vitro* clonal propagation was required for efficient multiplication. The explants were surface sterilized by pre-soaking in Topsin-M fungicide and sequential use of $HgCl_2$ and NaOCl. Phenolic exudation was controlled using 1% Citric acid solution. Among selected accessions, #33 performed better for efficient establishment of cultures and *in vitro* clonal multiplication. Hence, it may be used for further clonal propagation using tissue culture for commercial production. This is first report of the adoptability studies of different HT-Rose accessions in Pakistan and their capacity to be used in selection and breeding programs.
Key Words: Adoptability, Characterization, *In vitro*, Phenotype, Principal component analysis

INTRODUCTION

Rose (*Rosa indica* L.) of family *Rosaceae* is the most famous ornamental flower around the globe. Rose has extraordinary fragrance and beauty for landscaping and Cut-flower use. Rose contains extraordinary human health benefits. Different parts of plant used for healing skin injuries and rashes, anti-depression, and treatment of migraine (Cook, 2018). World largest producer of Rose is India (28130 ha.) followed by China (14316 ha.) and Ecuador (4073 ha.) (Hanks, 2015). Netherland (775.5 M Eu), Kenya (299.5 M Eu) and Ethiopia (136.3 M Eu) are leading exporters, while world largest importer of Rose is Netherlands (416.3 M Eu) too (Hanks, 2015). The international scenario of Rose production, export and import depicts that the Netherlands dominates the Rose market around the world. Rose is adopted in a wide range of variable climatic pockets from Hot to cold and dry to humid regions in Pakistan. Rose as fresh cut-flower is cultivated on 1500 acres in Pakistan (Riaz and Monis, 2017) which is negligible compared with other cash crops.

Hybrid Tea Roses are available in numerous colors and shades. The attributes like recurrent blooming, high centered bud, a greater number of petals/flower, variegated floral colors, good floral stem size, no thorns and disease escape are the most desirable characters of commercial varieties. Hybrid tea roses offer desirable attributes for diverse climate of Pakistan. Hybrid tea roses are pedigree of *Rosa gallica*, *Rosa moschata*, *Rosa chinensis* and hybrids of *Hybrid perpetual* × *Tea rose* (Gender, 1965). The hybrid rose varieties are found in triploid or tetraploid (Rout *et al.*, 1999) level. The different trends of Ploidy are main hurdle in viable seed formation, due to which they cannot be easily included in crop improvement programs. Hybrid roses did not form hip through cross pollination while self-pollination rarely support hip/seeds formation (Cairns, 2000), however in Pakistan seed formation in HT-Roses is rarely observed. The first step in crop improvement program is screening of germplasm either against stresses such as biotic, abiotic and quality attributes. Through selection more than 20,000 varieties are developed for various environmental and biological stresses through various crop improvement programs.

Pakistan is world's eight most vulnerable countries with climate change activities where unexpected rains and

droughts are prevailed (Akbar, 2018). Pakistan is facing climate change crises for the last 20 years. Pakistan estimates 3.8 billion dollars ($) due to climate change effects on economy through massive floods and droughts (Akbar, 2018). Growth potential of Rose is affected greatly by fluctuation in climatic attributes like temperature, humidity, and solar irradiation. The optimum temperature for best quality attributes is 15-28^{o}C while irregularities in temperature showed uneven and stagnant plant growth in rose during recent past around the tropical and sub-tropical regions (Blom and Tsujita, 2003). Rose is relatively hardy and adopted in each climate, however in unfavorable climate it works for survival mechanisms compromising better yield through Osmoregulation, Turgor adjustment and Osmopartition among apoplast and symplast (Mann, 2002).

Phenotypic plasticity of hybrid roses is the results of adoptability (Korir *et al*., 2012). Prior to use molecular markers it is necessary to identify the plants at phenotypic level for to observe variation. Morphology studies are phenotypic markers of plant identification and characterization. Distinguished varietal characters never change due to exposure of hard climatic and edaphic factors (Maddison, 1996). Morphological characterization is easy to understand and hence everyone can used to verify and study a certain genotype (Gaurave *et al*., 2018). The morphological markers in plant are indicators of many plant defense and tolerant mechanisms. Abundance of Anthocyanin pigments are indicator for high chemical activity for resistance against cold (Lev-Yadun 2006). While increased anthocyanin turns the leaves green into red. The red color become more visible to insects and increases the invasion. Evaluation and clustering comparison of species and accessions (mainly landraces) are helpful in establishing hereditary relationships along with pedigree of different crops in various regions around the globe. Morphological attributes are also important for efficient utilization of these accessions in breeding e.g. knowing the floral color and less thorn (Kaul *et al.,* 2009). Adequate characterization of introduced varieties is vital for efficient utilization of germplasm by recommendation for local population according to market demand. The results of morphological characterization are evenly useful for estimating genetic diversity amongst varieties and inbred lines (Ye *et al.,* 2008).

Followed by identification of desirable lines and accessions it is necessary to propagate them for further use. In vitro propagation is technique used for conservation and mass multiplication of elite germplasm of different plants (Murashige and Skoog, 1962). *In vitro* propagation provides year-round facility of plant production. It removes many hurdles and time consumption for different aspects of research especially against edaphic factors such as drought and salinity. Diversity studies accompanied with plant tissue culture propagation protocol can help to conserve and multiply the desirable verities with beneficial characters. The present study was carried out for evaluation of diversity amongst elite germplasm through morphological attributes and *in vitro* protocol establishment for superior strain multiplication for further research.

MATERIALS AND METHODS

***Morphological Diversity Analysis in Hybrid Rose Germplasm*:**

The present research was carried out during the year of 2017-2019 in Horticultural research institute for floriculture and landscaping, Islamabad (HRI). UPOV-Rose (*Rosa indica* L.) descriptor was used for morphological characterization of the 41 Rose accession collected during last 10 years from private nurseries of Lahore and Pattoki Punjab. Accession-1 (White), Accession 2-5 (Creamy), Accession 6-9 (Yellow), Accession 10-15 (Purple), Accession-16 (Orange), Accession 17-32 (Pink) and Accession 35-41 (Red) were subjected to study and maintain in germplasm block in HRI.

***Plant attributes for morphological diversity analysis*:** The morphological attributes studied were as Tr1 = Excluding varieties with growth type climber: Plant Growth habit; Tr2 = Plant Growth: Type; Tr3 = Plant: Height (during second flush); Tr4 = Young shoot anthocyanin colorations; Tr5 = Young shoot intensity of anthocyanin coloration; Tr6 = Stem: Number of prickles (Excluding very small and hair like prickles); Tr7 = Prickle: Pre dominant color; Tr8 = Leaf: Size; Tr9 = Leaf: intensity of green color (Upper Side); Tr10 = Leaf: Anthocyanin coloration (Young); Tr11 = Leaf: Glossiness of upper side; Tr12 = Leaflet: undulation of margin; Tr13 = Terminal leaflet: Shape of blade; Tr14 = Terminal leaflet: Shape of base of blade; Tr15 = Flowering shoot: Flowering laterals; Tr16 = Flowering shoot: Number of Flowering laterals; Tr17 = Only verities with no flowering laterals: Flowering shoot: Number of Flowers; Tr18 = Only varieties with flowering lateral: Flowering shoots: Number of flowers per lateral; Tr19 = Flower bud: shape in longitudinal section; Tr20 = Flower: Type; Tr21 = Flower: Number of petals; Tr22 = Flower color groups; Tr23 = Only varieties with flower type: double flower: Color of center; Tr24 = Only Variety of Flower Type: Double flower: Density of petals; Tr25 = Flower: Diameter; Tr26 = Flower: Profile of Upper Part; Tr27 = Flower: Profile of lower part; Tr28 = Flower: Fragrance; Tr29 = Sepals: Extension; Tr30 = Petals: Refluxing of petals one-by-one; Tr31 = Petals: Shape; Tr32 = Petals: incisions; Tr33 = Petals: Reflexing of margin; Tr34 = Petals: undulation; Tr35 = Petals: Size; Tr36 = Petals: Length; Tr37 = Petals: width; Tr38 = Petals: Number of colors on inner side of petals (Basal Spot exclude); Tr39 = Only Varieties with one color on inner side of petals: Petal: intensity of color (Basal Spot exclude); Tr40 = petals: Main Color on the inner side (main color is that with largest surface area); Tr41 = Only varieties with two or more color on inner side of petals: Petals: Petal secondary color (Basal spot exclude); Tr42 = Petal: Size of basal spot on inner side; Tr43 = Petal: Color of basal spot on inner side; Tr44 = Petal: main

color on the outer side (only if clearly different from inner side); Tr45 = outer stamen: predominant color of filament; Tr46 = Seed Vessel: Size (at petal fall); Tr47 = Hip: Shape in longitudinal section; Tr48 = Hip: Color (at mature stage); Tr49 = Sprouting time; Tr50 = Seed formation; Tr51 = Seed viability (UPOV, 2010).

In Vitro Propagation of Hybrid Rose Germplasm

***Plant material and sterilization procedures*:** The nodal explants were collected from selected plants of accessions 6, 18, 33 and 34. The explants were washed with running tap water for 1 hr. followed by soaking in Topsin-M. (0.5%) fungicide. Mercuric chloride (0.05%) for 3 minutes followed NaOCl (10%) and C_2H_5OH (70%) for 10 minutes were used for control of microbial contamination. The phenolic exudates were reduced by rinsing explants into ascorbic acid (10 mg/L) solution for 10 minutes. The cultures were placed into growth room at 18-22^oC with 16/8 hrs. light and dark conditions.

***Media formulations*:** Murashige and Skoog (1962) 'MS' media was used following Attia *et al.* (2012) and Ozel and Arslan (2006) and modified with TDZ (Thidiazuron) 0.5-1.0 mg/L, kinetin 0.2mg/L and NAA (1-Naphthalene acetic acid) 0.1 mg/L for shoot induction. Rooting media comprised MS media supplemented with IBA (Indole-3-butyric acid) @ 1.0, 1.5 and 2.0 mg/L and IAA (Indole-3-acetic acid) in the regenerated plantlets.

***Statistical analysis*:** The data were analyzed by computing the means of percentage of each attribute followed by Principal Component Analysis and correlation matrix. The results of the *in vitro* propagation were analyzed by calculating the means followed by ANOVA and least significant difference test (Steel *el al.*, 1996).

RESULTS

***Morphological diversity analysis in hybrid Rose germplasm*:** The qualitative and quantitative observations were subjected to statistical analysis separately. The qualitative observation was analyzed by Chi square while numeric data was analyzed through t-test. Morphological attributes of various plant characters were coded with numeric values and based on coding percentage of each attributes were calculated with in an accession. Once percentage was calculated out of total variation trait of dominated percentage were considered as accession characters e.g. in Accession-17 young shoots anthocyanin coloration were weak in 80% plant population under study, so presence of weak anthocyanin coloration was considered as characters of young shoots in Accession-17. The results presented in this study are dominated characters of population studied in Islamabad conditions (the supplementary data is available on demand) according to UPOV-Rose descriptors from Plant shape to flower hip coloration. Elite hybrid Accessions-1 to 5 was from white to creamy flower color group (Table 1). The accessions were shrub type in nature with many-few thorns on mature branches. Accession-2, accession-3 and accession-5 showed less number of prickles on mature branches. The flower petals were many and with medium or low intensity of fragrance. In accession-2 seed vessel was large with full of seeds however viability of seeds was not observed in subsequent seasons. Results at <0.05 accession-4 took least days (19.33) days for sprouting in spring followed by accession-5 selection (25.5). Accession-5 showed highest plant height (107cm) followed by accession-4 (101.20cm). Accession-6 to accession-9 observed yellow colored showed low intensity of flower fragrance. Accession-6 and accession-9 showed less prickles (table 1). Accession-6 showed least days for sprouting (21.76) followed by accession-7 (26.03). Highest plant heights were observed in accession-7 (121.0) followed by Accessuin-9 (82.47), while flower stick size was greater in Accession-8 (53.86). Shrub rose accession-10 to accession-16 produces purple or dark blue colored flowers in Islamabad conditions during 2017-2019 (Table 1). The purple accessions showed excessive thorns in stems having light to dark green colored leaves. The purple accessions showed strong fragrance while seed production was not been observed during the study since 2010. Accession-15 sprouted earlier (19.50) followed by accession-10 (20.53) after winter pruning for new flushes. Accession-16 produces longest stick size (48.0 cm) followed by accession-15 (42. 06cm). Highest plant height was showed by accession-13 (95.86cm) followed by accession-14 (93.13cm). Accession-17 to accession-32 was showed pink color (Table 1). Accession-26, accesion-29, accession-18 and accession-24 had low intensity of prickles. The accession with few surface prickles had strong to medium intensity of floral fragrance. Accession-24 sprouted in least days (12.265) followed by accession-24 (15.56) and accession-18 (16.37). Floral stick length was recorded highest (65.10cm) followed by accession-31 (61.567cm) while the highest floral diameter was also recorded in accession-18 (11.43cm) followed by accession-20 (11.43cm). Greater plant height was also recorded in accession-32 (117.77cm) followed by accession-19 (110.37cm). Accession-33 to accession-41 was of red color in HT-Rose with different intensity of thorns from few to many. Red color varieties including accession-33 reflect medium to weak intensity of fragrance (Table 1). Accession-39 took less days (14.967) for sprouting after winter pruning followed by accession-33 (17.43). Highest plant height was observed in multicolor accession-41 (110.63cm) followed by accession-34 (105.83cm), while good floral stick size was observed in accession-39 (53.07cm) and accession-33 (50.70cm).

Table 1. Morphological attributes of hybrid Rose accessions collected from various geographical locations of Punjab.

Accession Sr.	Flower Color	Seed formation	Days for sprouting after autumn pruning	Plant Height (cm)	Floral Stick length (cm)	Flower Diameter (cm)
1	White	-	23.01bc	88.53b	28.90bc	6.96c
2	Creamy	+	26.06a	73.80c	42.87a	12.06a
3	Creamy	-	32.01a	81.97b	25.60c	9.20abc
4	Creamy	-	19.33c	101.20a	30.46bc	8.60bc
5	Creamy	-	25.01b	107.00a	37.33ab	10.30ab
6	Yellow	-	21.76c	82.97c	37.26c	8.26c
7	Yellow	-	26.03b	121.00a	41.63b	12.93a
8	Yellow	-	30.56a	94.13b	53.86a	10.76b
9	Yellow	-	33.37a	82.47c	43.23b	6.90c
10	Purple	-	20.53d	76.70b	38.83b	6.50c
11	Purple	-	26.16c	90.10a	42.03b	8.56b
12	Purple	-	22.26d	87.43a	31.86c	10.96a
13	Purple	-	28.43bc	95.86a	39.44b	8.40b
14	Purple	-	30.33ab	93.13a	34.16c	10.50a
15	Purple	-	19.50d	55.80c	42.06b	5.90c
16	Orange	-	32.50a	49.56c	48.00a	5.46c
17	Pink	-	22.30cd	91.37de	40.13ef	6.76e
18	Pink	+	16.36ef	95.20cd	65.10a	11.50a
19	Pink	-	27.00bc	110.37b	59.90b	9.50abcd
20	Pink	-	29.03ab	66.03hi	43.06de	11.43ab
21	Pink	+	25.30bcd	75.70fg	22.33h	8.53cde
22	Pink	-	15.56f	69.57hi	19.66h	10.10abc
23	Pink	-	21.26de	96.13cd	42.73def	9.83abcd
24	Pink	-	12.26f	78.17f	39.63f	10.26abc
25	Pink	-	32.90a	70.73gh	42.60def	9.26cd
26	Pink	-	26.20bcd	80.30f	32.23g	7.75de
27	Pink	-	24.03bcd	88.23e	41.40def	8.60cde
28	Pink	-	32.83a	67.37hi	34.60g	8.26cde
29	Pink	-	28.63ab	97.00cd	55.26c	8.66cde
30	Pink	-	25.33bcd	64.00i	43.60d	8.53cde
31	Pink	-	25.33bcd	100.80c	61.56b	9.30bcd
32	Maroon	-	25.30bcd	117.77a	41.60def	9.06cd
33	Red	-	17.43ef	90.03c	50.70ab	8.83a
34	Red	-	29.73a	105.83a	35.30d	8.13a
35	Red	-	24.76bc	83.10d	33.06d	7.667a
36	Red	-	28.80a	95.53bc	45.76c	8.23a
37	Red	-	22.66cd	79.67d	48.50bc	8.23a
38	Red	-	27.53ab	68.90e	44.76c	9.23a
39	Red	-	14.96f	96.23b	53.06a	8.66a
40	Red	-	24.66bc	90.13c	35.80d	10.06a
41	Multi-color	-	20.36de	110.63a	52.86ab	9.40a

NS=Non-significant (P>0.05); *=Significant (P<0.05); **= highly significant (P<0.01); SD = Standard deviation, SE = Standard error

***Principal component analysis (PCA) based on dissimilarity in hybrid Rose traits and accessions*:** Principal component analysis was carried out to identify multidimensional relationship amongst different varieties for grouping traits and varieties for sharing variation in the gene pool. The PCA extracts all important variables in the form of different components from a large set of variables available in data set and show in more interpretable form. The traits studied according to morphological descriptors were projected on the factor plan (Factor1 Vs Factor2) where collective response of the varieties against Tr1 (Exclusive varieties with growth climber), Tr2 (Plant: Growth type), Tr3 (Plant: Height), Tr4 (Young shoot anthocyanin coloration), Tr5 (Young shoot intensity of anthocyanin coloration), Tr6 (Stem: Number of Prickles), Tr7 (Prickle: Predominant coloration, Tr9 (intensity of green color), Tr15 (Flowering shoots: flowering laterals),

Tr16 (Flowering Shoots: Number of flowering laterals), Tr24 (Flowers: Density of Petals), Tr25 (Flower: Diameter), Tr29 (Sepals: Extension), Tr30 (Reflex of petals one by one), Tr31 (Petals: Shape), Tr32 (Petals: Incision) and Tr33 (Petals: Reflex of Margins), Tr 41 (verities with two or more color on inner side) contain 11.43 variation as shown in the (Fig. 1) while the rest of the attributes studied contain 10. 14% of variation. Rose varieties on Projection of cases in factor plane (Factor1×Factor2) showed narrow range variation 11.43% by PCA in accession-17, accession-2, accession-19, accession-20, accession-10 and some other (Fig. 3) while rest of the accession contain 10.14% of variation.

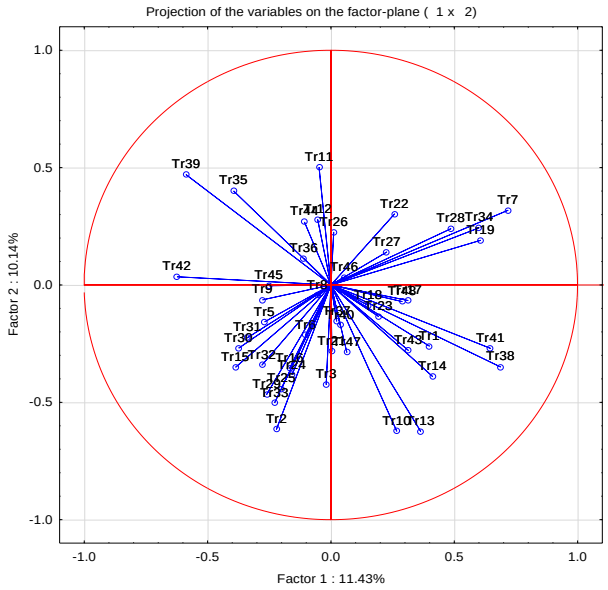

Figure 1. Distribution of observed traits on factor plain (Factor1×Factor2).

Tr1 = Excluding varieties with growth type climber: Plant Growth habit; Tr2 = Plant Growth: Type; Tr3 = Plant: Height (during second flush); Tr4 = Young shoot anthocyanin colorations; Tr5 = Young shoot intensity of anthocyanin coloration; Tr6 = Stem: Number of prickles (Excluding very small and hair like prickles); Tr7 = Prickle: Pre-dominant color; Tr8 = Leaf: Size; Tr9 = Leaf: intensity of green color (Upper Side); Tr10 = Leaf: Anthocyanin coloration (Young); Tr11 = Leaf: Glossiness of upper side; Tr12 = Leaflet: undulation of margin; Tr13 = Terminal leaflet: Shape of blade; Tr14 = Terminal leaflet: Shape of base of blade; Tr15 = Flowering shoot: Flowering laterals; Tr16 = Flowering shoot: Number of Flowering laterals; Tr17 = Only verities with no flowering laterals: Flowering shoot: Number of Flowers; Tr18 = Only varieties with flowering lateral: Flowering shoots: Number of flowers per lateral; Tr19 = Flower bud: shape in longitudinal section; Tr20 = Flower Type; Tr21 = Flower: Number of petals; Tr22 = Flower color groups; Tr23 = Only varieties with flower type: double flower: Color of center; Tr24 = Only Variety of Flower Type: Double flower: Density of petals; Tr25 = Flower: Diameter; Tr26 = Flower: Profile of Upper Part; Tr27 = Flower: Profile of lower part; Tr28 = Flower: Fragrance; Tr29 = Sepals: Extension; Tr30 = Petals: Refluxing of petals one-by-one; Tr31 = Petals: Shape; Tr32 = Petals: incisions; Tr33 = Petals: Reflexing of margin; Tr34 = Petals: undulation; Tr35 = Petals: Size; Tr36 = Petals: Length; Tr37 = Petals: width; Tr38 = Petals: Number of colors on inner side of petals (Basal Spot exclude); Tr39 = Only Varieties with one color on inner side of petals: Petal: intensity of color (Basal Spot exclude); Tr40 = petals: Main Color on the inner side (main color is that with largest surface area); Tr41 = Only varieties with two or more color on inner side of petals: Petals: Petal secondary color (Basal spot exclude); Tr42 = Petal: Size of basal spot on inner side; Tr43 = Petal: Color of basal spot on inner side; Tr44 = Petal: main color on the outer side (only if clearly different from inner side); Tr45 = outer stamen: predominant color of filament; Tr46 = Seed Vessel: Size (at petal fall); Tr47 = Hip: Shape in longitudinal section; Tr48 = Hip: Color (at mature stage); Tr49 = Sprouting time; Tr50 = Seed formation; Tr51 = Seed viability

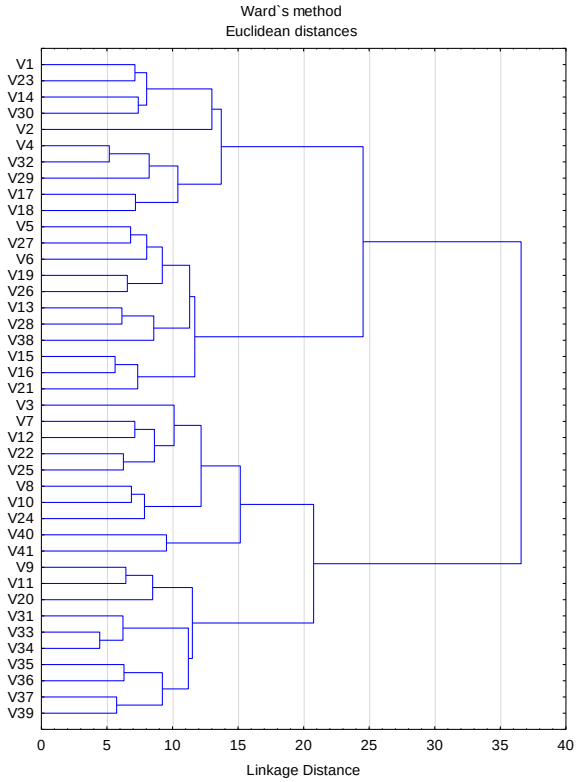

Figure 2. Dendrogram based on the hierarchical clustering of Elite HT-Rose accessions.

Where V1 = Accession-33; V2 = Accession-6; V3 = Accession-17; V4 = Accession-34; V5 = Accession-18; V6 = Accession-2; V7 = Accession-10; V8 = Accession-19; V9 = Accession-20; V10 = Accession-7; V11 = Accession-11; V12 = Accession-12; V13 = Accession-21; V14 = Accession-22; V15 = Accession-23; V16 = Accession-32; V17 = Accession-9; V18 = Accession-13; V19 = Accession-3; V20 = Accession-35; V21 = Accession-16; V22 = Accession-25; V23 = Accession-14; V24 = Accession-01; V25 = Accession-29; V26 = Accession-4; V27 = Accession-30; V28 = Accession-31; V29 = Accession-36; V30 = Accession-27; V31 = Accession-28; V32 = Accession-37; V33 = Accession-26; V34 = Accession-15; V35 = Accession-38; V36 = Accession-21; V37 = Accession-24; V38 = Accession-39; V39 = Accession-40; V40 = Accession-8; V41 = Accession-41

Cluster analysis of hybrid rose germplasm

Hierarchical cluster analysis of HT-Rose cultivars planted in Islamabad conditions was carried out through "Ward D clustering" and a dendrogram was constructed. The HT-Rose cultivars were divided into five groups (Figure 2) at linkage distance of 10. In first cluster accession-33, accession-22, accession-14 and accession-27 were included while in cluster second accession-6, accession-34, accession-37, accession-36 and accession-9. The third group is the largest group of similar genotypes included accession-18, accession-30, accession-2, accession-3, accession-12, accession-15, accession-39, accession-23, accession-5 and accession-16. The fourth group included accession-17, accession-10, accession-12, accession-25, accession-29, accession-19, accession-7, accession-1, accession-8 and accession-41 at the ward's Distance of 10. Ward's linkage distance at 5 clusters fifth include accession-20, accession-11, accession-35, accession-28, accession-26, accession31, accession-38, accession-21, accession-24 and accession-32.

Group one and group two have linkage distance of 25 while group three and four fall in group distance 20. Further, more the cluster analysis using MSS package of R, and the relationship between different clusters is given in table 2. The HT-Rose accessions subjected to observation are from different source and countries, cluster analysis revealed that this germplasm have a wide range of variation for including them in crop improvement program against different edaphic factors.

Table 2. Membership score of different clusters of the dendrogram using linear discriminant analysis.

	Cluster				
Cluster	**1**	**2**	**3**	**4**	**5**
1	4	0	0	0	0
2	0	6	0	0	0
3	0	0	11	0	0
4	0	0	0	10	0
5	0	0	0	0	10

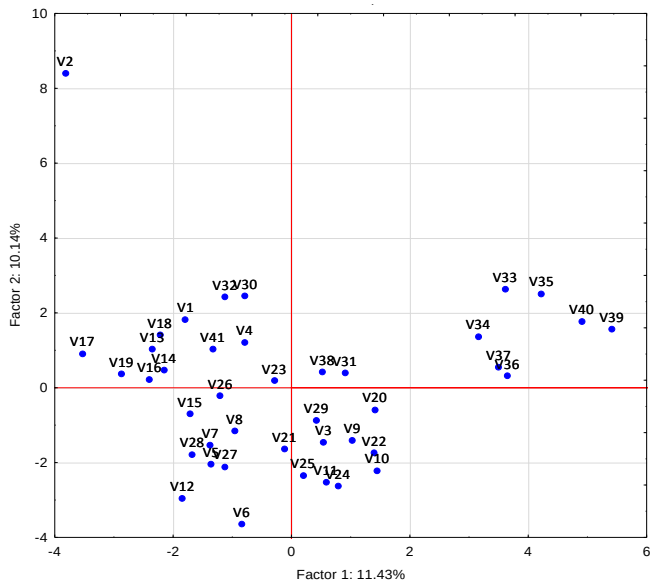

Figure 3. Principal component analysis PCA based on the dissimilarity index of HT-Rose accessions.

Where V1 = Accession-33; V2 = Accession-6; V3 = Accession-17; V4 = Accession-34; V5 = Accession-18; V6 = Accession-2; V7 = Accession-10; V8 = Accession-19; V9 = Accession-20; V10 = Accession-7; V11 = Accession-11; V12 = Accession-12; V13 = Accession-21; V14 = Accession-22; V15 = Accession-23; V16 = Accession-32; V17 = Accession-9; V18 = Accession-13; V19 = Accession-3; V20 = Accession-35; V21 = Accession-16; V22 = Accession-25; V23 = Accession-14; V24 = Accession-01; V25 = Accession-29; V26 = Accession-4; V27 = Accession-30; V28 = Accession-31; V29 = Accession-36; V30 = Accession-27; V31 = Accession-28; V32 = Accession-37; V33 = Accession-26; V34 = Accession-15; V35 = Accession-38; V36 = Accession-21; V37 = Accession-24; V38 = Accession-39; V39 = Accession-40; V40 = Accession-8; V41 = Accession-41

The morphological attributes and PCA showed narrow range of variation amongst the elite HT-Rose accession in general while some attributes of economic importance are observed in leading accessions such as less prickles in each of color group such as from white to Creamy color group accession-2, accession-3, while accession-6, accession-18 and accession-33 are from yellow, Pink and red color group respectively, while these genotypes also produces good cut-flower stick size compared with other genotypes along with normal plant height. These indices can also be indicators of leading genotypes for selection of desirable genotypes for further breeding programs.

Figure 1. Hybrid Rose accessions of various color shades studied and planted in the Germplasm unit (GPU).

***In vitro propagation of hybrid rose accessions*:** Micropropagation is the most necessary step in any crop

Table 3. Effect of growth regulators on in vitro shoot regeneration of hybrid Rose accessions

Attributes	Plant growth regulators	Accession-33	Accession-34	Accession-6	Accession-18
Days to sprouting	TDZ	13.67±0.65A	7.33±0.60B	11.67±1.03A	8.67±0.87B
	Kinetin	9.67±0.86B	9.67±0.99B	9.00±0.87B	14.00±0.84A
	BAP	8.67±0.87B	8.33±0.99B	12.33±0.90A	10.67±0.60AB
Number of Shoots	TDZ	3.44±0.25AB	2.94±0.17B	4.06±0.26A	4.11±0.21A
	Kinetin	3.56±0.17A	3.94±0.17A	4.06±0.22A	3.72±0.16A
	BAP	4.44±0.25A	3.11±0.16B	3.72±0.16AB	3.61±0.22B
Shoot Length (mm)	TDZ	4.85±0.21B	6.24±0.25A	6.07±0.29A	4.94±0.28B
	Kinetin	6.00±0.23B	6.91±0.30A	6.13±0.22B	6.58±0.27AB
	BAP	6.00±0.23AB	6.83±0.23A	5.65±0.19B	6.04±0.27AB
Survival %age	TDZ	64.44±3.15A	50.56±2.21BC	58.33±4.22AB	38.89±2.54C
	Kinetin	55.56±2.17A	49.44±2.49AB	40.00±1.98C	41.67±2.46BC
	BAP	56.67±2.91A	46.11±2.16AB	43.33±2.43B	56.67±3.88A

NS=Non-significant (P>0.05); *=Significant (P<0.05); **= highly significant (P<0.01); SD = Standard deviation, SE = Standard error

Table 4. Effect of IBA on root growth attributes in hybrid Rose accessions

Attributes	Accession-33	Accession-34	Accession-6	Accession-18
Days to root induction from regenerated shoots	12.33±1.13A	10.00±0.84A	9.33±0.87A	10.33±0.65A
Number of roots	3.06±0.21B	4.17±0.22A	5.00±0.27A	4.28±0.19A
Root length (mm)	5.64±0.21A	5.62±0.17A	5.78±0.26A	6.02±0.22A

NS=Non-significant (P>0.05); *=Significant (P<0.05); **= highly significant (P<0.01); SD = Standard deviation, SE = Standard error

mprovement program for conservation and genetic manipulation of desirable genotypes

Figure 2. Micropropagation of rose accessions where: A-plant population from where explants were collected, B-explants after excision and sterilization, C-cultures with sprouted nodes in culture vessel, D-plants in acclimatization phase in the laboratory.

Elite varieties accession-33, accession-34, accessoion-6 and accession-18 were subjected to *in vitro* propagation. TDZ, Kinetin and different doses of BAP were used for better shoot induction as well. The use of growth regulators enhanced early explant sprouting, shoot length (mm), number of shoots and had better plant survival compared with control (Table 3). The multiplied shoots were cultured in root induction medium for rooting and rooted plants were transferred to field after acclimatization. Accession-33 (8.33) and accession-34 (8.67) took least days for explant sprouting. More number of shoots (4.44) and higher shoot length (6.00) was observed in accession-33 on BAP. The highest plant survival was observed in accession-33 on TDZ (64.44) compared with rest of the varieties and growth regulators. Addition of IBA showed good root length in accession-33 (5.64) while a greater number of roots was observed in accession-6.

DISCUSSION

Characterization of hybrid Rose germplasm through morphological markers: Number of Morphological studies has been carried out in Rose (Wissemann, 2003) for characterization. However, adoptability and performance in distinct regions is a complex phenomenon because of their different ploidy number and spontaneous variation due to cross pollination (Gudin, 2000). The Study of Morphological attributes with respect to adaptability have prime importance in various breeding programs against different soil and edaphic level. Morphological relationships of varieties remain highlighted due to complex taxonomic variability (Singh, 2017) in rose germplasm. There is vast gene pool of hybrid rose varieties in Pakistan planted since long. Due to spontaneous mutation and chance seedlings number of local

varieties and selections are introduced. Many of HT-Rose accessions are performing excellently in different edaphic zones. Morphological markers are taxonomic markers used for identification and characterization of genotypes for further breeding programs against different edaphic factors. Morphological basis of plant characters showed narrow variation amongst different varieties. However, some elite accessions showed distinct behavior in less intensity of prickles, and high anthocyanin coloration at both young and mature shoot stage (Landi *et al.*, 2015). However, intra and inter accession variation was observed almost in all characters (supplementary data). HT-Rose accessions showed upright and shrub type of growth which can be utilized for bed plantation. Such type of genotypes can used in the crop improvement programs related to evolving new strains through physical mutation against edaphic factor such as drought and salinity tolerance. In Rose thrones are known as prickles which do not have vascular bundles and can easily be removed. They are present in various densities and sizes on various rose cultivars planted (Debener and Linde, 2009). Thorn less varieties are always desirable for effective conventional and non-conventional breeding programs (Kanli and Kazaz, 2009; Zlesak, 2007), the elite accession-33, accessions-34, accessions-6, accession-18 and accession-2 showed less prickles compared with others. Prickliness is manipulated by the recessive alleles while shoot prickles high intensity is controlled by dominated alleles. The varieties which showed high intensity of prickles have dominated alleles. In Islamabad climatic conditions most of Hybrid cultivars did not form seeds having small to medium size hip formation. Abortion of hip said to be due to abrupt high temperature (45^{o}C) during start of summer and late spring while in Islamabad the mean spring temperature in lower than 30^{o}C (Visser *et al.*, 1977) due this lack of seed formation due to high temperature is rejected. However, it is possible that seed formation my effect by internal physiological processes during pollination and fertilization (Gudin, 1992) however some varieties e.g., accessions-2, accession-18 and accession-21 formed large hip and seed formation in subsequent days. Lack of seed formation is may be due multiple genetic factors alongside with edaphic factors such as lethal genes and its deleterious effects, mitotic abnormalities, self or cross incompatibility, variability in ploidy and interspecific factors (Ogilvie *et al*., 1991). Variation was also observed in germplasm in Islamabad geographical conditions, while floral size is directly influenced by presence of a greater number of whorls however climatic conditions also affect the number of flowers and flower diameter which is affected by humidity level as high humidity is observed in flowering season in Islamabad.

***Efficient in vitro propagation of selected hybrid Rose accessions*:** The *in vitro* propagation is tough in roses due to phenolic control and inborn contamination (Pati *et al.*, 2006). The numeric data is shown in the form of representative and collective mean of growth regulators; however, supplementary data is available in record for detail treatment response of growth regulator applied for shoot and root induction. Sterilization protocol used during this study was confirmed by Rout *et al.,* (1989) for Rose shoot induction, however, use of fungicide helped to avoided contamination in cultures. Browning of medium is from the cut surface of explants of HT-accession was due to the phenolic substances present in the plants. These exudates were controlled by agitating of explants into low dose of citric acid solution. The citric acid is antioxidant which remove phenolic substances from surface of explants and allow transfusion of media nutrients in explants for completion of growth activities (Rout *et al.*, 1999). Significantly better response of accession-33 in *in vitro* conditions is also evident (Horn, 1992). The rooting from regenerated shoots were observed in MS-Medium modified with different doses of IBA and NAA. The IBA induced significant number of roots while no significant difference between different doses of IBA was observed for root induction (Table 4).

***Conclusion*:** Morphological diversity assessment is key factor in identifying potential genotypes for further use in breeding programs. There is need to subject the material to molecular analysis for further selection of material against biotic and abiotic stress. In Pakistan, a huge amount of capital is invested for import of exotic flora for landscape and home gardening. Identifying the potential genotypes and further breeding will help to save capital invested in import, moreover low-price and high-quality commodity will also be available for the local consumers. Selection for crop improvement can be mobilized for larger economic sector for foreign capital earning. Pakistan has the potential to emerge as potential exporter of floral products to near and distinct markets.

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